DEPARTMENT OF HEALTH AND HUMAN SERVICES

Nomination of In Vitro Test Methods for Detection and Quantification of Botulinum Neurotoxins and Detection of Non-Endotoxin Pyrogens; Data Request for Substances Evaluated by These Test Methods

AGENCY: Division of National Toxicology Program (NTP), National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH).

ACTION: Request for comments and/or data.

SUMMARY: On behalf of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) requests public comment on nominations received for (1) Three *in vitro* test methods proposed for detecting and quantifying botulinum neurotoxin (BoNT), and (2) an *in vitro* test method proposed for detecting non-endotoxin pyrogens. NICEATM seeks data generated using alternative test methods for detecting and quantifying BoNT, including but not limited to three test methods nominated by BioSentinel Pharmaceuticals, Inc. (BioSentinel). Data from the standardized mouse LD_{50} assay currently used for these endpoints are requested for comparison. In addition, NICEATM seeks data generated using alternative test methods for identifying non-endotoxin pyrogens, including but not limited to the monocyte activation test (MAT), which was nominated by Biotest AG. Data on non-endotoxin pyrogens tested in the rabbit pyrogen test (RPT) are requested for comparison. NICEATM received nominations for validation studies on each of the above test methods, which have the potential to reduce or replace animal use for regulatory testing. At this time, ICCVAM requests public comments on the appropriateness and relative priority of these activities.

DATES: For consideration by the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) at its annual meeting (67 FR 23323), comments and data are

requested by June 2, 2011. NICEATM and ICCVAM will accept comments and data for these nominations until July 7, 2011.

FOR FURTHER INFORMATION CONTACT: Dr. Warren Casey, Deputy Director, NICEATM, NIEHS, P.O. Box 12233, *Mail Stop:* K2–16, Research Triangle Park, NC, 27709, (telephone) 919–541–2384, (fax) 919–541–0947, (e-mail) *niceatm@niehs.nih.gov*. *Courier address:* NICEATM, NIEHS, Room 2034, 530 Davis Drive, Morrisville, NC 27560.

SUPPLEMENTARY INFORMATION:

Nomination for the Detection and Quantification of BoNTs

In 2006, NICEATM and ICCVAM convened a workshop, *Alternative Methods to Refine, Reduce, or Replace the Mouse LD_{50} Assay for Botulinum Toxin Testing,* in response to a nomination from the Humane Society of the United States requesting that ICCVAM assess the availability of alternative methods to replace the mouse LD_{50} assay for BoNT potency testing. Workshop participants concluded that some of the methods considered could be used, in specific circumstances or in a tiered-testing strategy, to reduce or refine the use of mice in current *in vivo* BoNT testing protocols (ICCVAM, 2008a). However, none of the reviewed methods was considered suitable to serve as a complete replacement for the mouse LD_{50} assay, either for detection of BoNT or for potency determination. The workshop participants noted that some of the methods considered might be useful as replacements for the mouse LD_{50} assay in the future given additional development and validation efforts.

BioSentinel has developed tests for the detection and quantification of BoNTs. These tests include the *in vitro* BoTest™ and BoTest™ Matrix assays and the cell-based assay BoCell™. Following appropriate validation and demonstration of adequate performance, these methods may have the potential to meet regulatory requirements for detection and quantification of BoNTs in a range of applications.

BioSentinel has forwarded a nomination for these methods to (1) Facilitate collaboration to develop a validation strategy which could lead to the regulatory acceptance of the test methods for the detection and quantification of BoNT contained in suspect substances, the determination of drug product potency, and/or the clinical diagnosis of botulism and (2) coordinate and conduct necessary validation studies.

Nomination for the Detection of Non-Endotoxin Pyrogens

ICCVAM previously evaluated the validation status of five *in vitro* test methods proposed for assessing the potential pyrogenicity (*i.e.,* ability to induce fever) of pharmaceuticals and other products, as potential replacements for the RPT. Subsequent to this evaluation, ICCVAM recommended that, although none of the test methods should be considered as a complete replacement for the RPT for the detection of Gram-negative endotoxin, they can be considered for use to detect Gram-negative endotoxin in human parenteral drugs on a case-by-case basis, subject to product-specific validation to demonstrate equivalence to the RPT, in accordance with applicable U.S. Federal regulations (ICCVAM, 2008b). ICCVAM recognized that these test methods could be applicable for detection of a wider range of pyrogens, including non-endotoxin pyrogens, and made recommendations for future studies that could expand their applicability. In response to these recommendations, Biotest AG recently nominated a commercialized version of one of these tests (*i.e.,* MAT), which uses cryopreserved human blood and quantitates the induction of interleukin (IL)-1β, for additional validation studies to evaluate its usefulness for identifying non-endotoxin pyrogens.

Draft ICCVAM Conclusions and Recommendations

Based on the information provided by the test method sponsors, ICCVAM concludes that the nominated activities are of sufficient interest and applicability to warrant further evaluation. ICCVAM's preliminary recommendation is that both nominations should have a high priority for further discussion to assess what information is needed to adequately characterize the usefulness and limitations of the proposed test methods, and any other similar *in vitro* test methods, for these endpoints. These assessments will identify what data are needed and what studies are required to fill any data gaps that are identified. Studies identified as necessary to adequately characterize the validation status for regulatory testing purposes are proposed to have a high priority.

As part of the nomination review process, NICEATM invites public comments on these nominations and the appropriateness and relative priority assigned by ICCVAM to the nominated activities. ICCVAM will finalize its recommendations on the priority of these nominations after considering comments received from the public and SACATM, which will comment on the ICCVAM draft recommendations at its meeting on June 16–17, 2011.

Background Information on ICCVAM, NICEATM, and SACATM

ICCVAM is an interagency committee composed of representatives from 15 Federal regulatory and research agencies that require, use, generate, or disseminate toxicological and safety testing information. ICCVAM conducts technical evaluations of new, revised, and alternative safety testing methods with regulatory applicability and promotes the scientific validation and regulatory acceptance of toxicological and safety testing methods that more accurately assess the safety and hazards of chemicals and products and that reduce, refine (decrease or eliminate pain and distress), or replace animal use. The ICCVAM Authorization Act of 2000 (42 U.S.C. 285l-3) established ICCVAM as a permanent interagency committee of the NIEHS under NICEATM. NICEATM administers ICCVAM, provides scientific and operational support for ICCVAM-related activities, and conducts independent validation studies to assess the usefulness and limitations of new, revised, and alternative test methods and strategies. NICEATM and ICCVAM work collaboratively to evaluate new and improved test methods and strategies applicable to the needs of U.S. Federal agencies. NICEATM and ICCVAM welcome the public nomination of new, revised, and alternative test methods and strategies for validation studies and technical evaluations. Additional information about ICCVAM and NICEATM can be found on the NICEATM–ICCVAM Web site (*http://iccvam.niehs.vnih.gov).*

SACATM was established in response to the ICCVAM Authorization Act (Section 285l–3(d)) and is composed of scientists from the public and private sectors. SACATM advises ICCVAM, NICEATM, and the Director of the NIEHS and NTP regarding statutorily mandated duties of ICCVAM and activities of NICEATM. SACATM provides advice on priorities and activities related to the development, validation, scientific review, regulatory acceptance, implementation, and national and international harmonization of new, revised, and alternative toxicological test methods. Additional information about SACATM, including the charter, roster, and records of past meetings, can be found at *http://ntp.niehs.nih.gov/go/167.*

References

ICCVAM. 2008a. ICCVAM–NICEATM/ECVAM Scientific Workshop on Alternative Methods to Refine, Reduce, or Replace the Mouse LD_{50} Assay for Botulinum Toxin Testing. NIH Publication No. 08–6416. Research Triangle Park, NC: NIEHS. *Available: http://iccvam.niehs.nih.gov/docs/biologics-docs/BoNTwkshprept.pdf.*

ICCVAM. 2008b. ICCVAM Test Method Evaluation Report: Validation Status of Five *In Vitro* Test Methods Proposed for Assessing Pyrogenicity of Pharmaceuticals and Other Products. NIH Publication No. 08– 6392. Research Triangle Park, NC: NIEHS. *Available: http://iccvam.niehs.nih.gov/methods/pyrogen/pyr_tmer.htm.*

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John R. Bucher,

Associate Director, National Toxicology Program.

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